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A Case—control Study on the Relationship between *Mycoplasma genitalium* Infection in Women with Normal Pregnancy and Spontaneous Abortion using Polymerase Chain Reaction

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Abstract

Objectives: *Mycoplasma genitalium* infections are suggested as causes of a number of pathological outcomes in pregnant women. The aim of this study was to evaluate the frequency of *M. genitalium* infections among pregnant women and its association with spontaneous abortion.

Methods: In this case—control study we included 109 women with spontaneous abortion with a gestational age of 10–20 weeks (patients), and 109 women with normal pregnancy with a gestational age of 20–37 weeks (controls) in Sanandaj, Iran. Using specific primers and extracted DNA from endocervical swabs, a polymerase chain reaction was conducted for the detection of *M. genitalium* infection in both groups.

Results: The frequency of *M. genitalium* infection in patient and control groups was one (0.91%) and three (2.75%), respectively. In both control and patient groups using Fisher test, no association between mycoplasma infection and spontaneous abortion was seen.

Conclusion: *M. genitalium* may be positive in the genital tract of some pregnant women but was not associated with spontaneous abortion. Further

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powerful studies with larger sample sizes are needed for the determination of a possible role of *M. genitalium* in pregnancy outcomes and spontaneous abortion.

1. Introduction

Spontaneous abortion prior to 20 weeks is a common adverse outcome of pregnancy, occurring in up to 15% of clinically recognized pregnancies. Usually spontaneous abortions occur in the first trimester due to phenotypic and/or chromosomal abnormalities, while environmental factors may have a greater impact on spontaneous abortion in later months of pregnancy [1]. Evidence suggests that maternal genitourinary and intrauterine infections have been proposed as an etiology for complications in pregnancy [2,3]. Mycoplasmas including *Mycoplasma genitalium*, *Mycoplasma hominis*, and *Ureaplasma* spp. are causes of sexually transmitted diseases (STDs) in men and women worldwide. About 80% of infected women and 50% of infected men may be asymptomatic [4]. *M. genitalium* and *M. hominis* are suggested as causes of a number of pathological outcomes in pregnant women. *M. hominis* was isolated from the amniotic fluid in 30% of women with intra-amniotic infection and was shown to be associated with preterm birth. *M. genitalium* is suggested to cause urethritis in men, mucopurulent cervicitis in women, and as an etiology of pelvic inflammatory disease independent of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* [1,5]. In addition to cervicitis, it has been detected in confirmed salpingitis and endometritis.

The most common pathway of intrauterine infection is vaginal and cervical infections and ascending route to amniotic cavity [6]. In addition, coinfection of mycoplasmas with other bacteria in the maternal genital tract during early gestation or preconception has been associated with spontaneous preterm birth, preterm premature rupture of the membranes, prematurity, spontaneous abortion, and perinatal morbidity and mortality [7–9]. Although mycoplasmas have been associated with these morbidities, the exact role of the *M. genitalium* in the etiology of spontaneous abortion has not been determined because of the difficulty in culturing it from clinical samples. Thus, association between mycoplasma infection and pregnancy outcome seems to be more reliable due to the development of sensitive molecular diagnostic methods such as polymerase chain reaction (PCR) [10]. In a study in Brazil, Campos et al [11] using quantitative PCR in 302 women showed that *M. hominis* and *M. genitalium* were detected in 31.8% and 28.1% of samples, respectively. Ahmadi et al [12], in a case–control study in Iran on 109 women with spontaneous abortion (patients) and 109 women with normal pregnancy (controls) using PCR reported that in patients the rate of *C. trachomatis* infections was 22.9% and 11.9%

in control. The association between *C. trachomatis* infection and spontaneous abortion had statistically significant. Hitti et al [13], in a case–control study in Peru using culture medium reported that in 661 cases with spontaneous preterm birth and 667 controls with normal delivery, *M. genitalium* was detected in 3% of them and was not a risk factor for preterm birth. Developed countries have established a national program for prenatal screening for infections in women, but in hospitals and clinics of Iran, there is such no national program [14]. The consequences of *M. genitalium* infection in pregnancy adverse outcome are unknown. The aim of this case–control study was to evaluate the frequency of *M. genitalium* infections among two groups of pregnant women (normal pregnancy and spontaneous abortion) and its association with spontaneous abortion.

2. Material and methods

2.1. Participants

This case–control and observational study was conducted in 2013. Pregnant women, attending the participating midwifery practices in the obstetrics and gynecology section or prenatal clinic and expected to deliver, were eligible for the study in Beasat Hospital, Sanandaj, Iran. We included 109 women (age, 19–43 years) with spontaneous abortion with a gestational age of 10–20 weeks (patients), and 109 (aged 19–43 years) women with normal pregnancy with a gestational age of 20–37 weeks (controls). Both groups included 26 women older than 25 years and 83 women younger than 25 years. Women expressed their informed consent to participate in this study. Demographic information such as age, education status, place of residence, occupation, and obstetrical data such as the number of childbirths, miscarriage, premature delivery, genital infection, urinary infection, the gestational age, and contraceptive methods before pregnancy were asked in a questionnaire and by a gynecologist. Inclusion criteria were having sexual activity and no use of antibiotics 2 weeks before sampling. Exclusion criteria were immunodeficiency, persons with chronic diseases (diabetes, endocrine disorders, and hypertension), recurrent miscarriage due to anatomic injury, and positive tests of first and second screening tests. In addition to asking when the 1st day of last menstruation was, ultrasound scan tests were done to estimate gestational age. To eliminate the threat of chromosomal abnormalities and probability of a genetic miscarriage, fetal health assessment tests were done at 11–13 weeks of gestation,

including nuchal translucency, double tests such as pregnancy-associated plasma protein A and free β -human chorionic gonadotropin. In addition, at 15–17 weeks of gestation the confirmatory triple tests were performed for elimination of probable neural tube and chromosomal anomalies. After the matching of the two groups (by gestational age and place of residence) and completing the questionnaire, participants were asked for two endocervical secretion samples using a cotton swap. Samples were then immediately taken into sterile 15-mL falcon tubes containing 5 mL of phosphate-buffered saline and placed at -70°C until DNA extraction. This study had the written consent of all its participants in both groups and was approved by the Ethics Committee of the Kurdistan University of Medical Sciences.

2.2. DNA extraction

Tubes containing cervical swab specimens were transported to the laboratory's compliance with the cold chain. Tubes were centrifuged at 6,000 rpm for 30 minutes. Then, the supernatant was discarded and the sediment was poured into the 1.5-mL microtube. The sediment was used for DNA extraction. DNA was extracted from sediments using a DNA extraction kit (High pure PCR Template Preparation; Roche, Germany) according to the manufacturer's instruction. After DNA extraction, to avoid degradation, the sample was split into separate 0.2-mL microtubes and maintained at -70°C until PCR diagnostic test.

2.3. PCR test for *M. genitalium* detection

We designed specific primers for the adhesion protein gene of *M. genitalium*, genome sequence deposited in

GeneBank. The *M. genitalium* primer sequences were: Forward: 5'-AGT TGA TGA AAC CTT AAC CCC TTG G-3' and Reverse: 5'-CCG TTG AGG GGT TTT CCA TTT TTG C-3'. The length of the *M. genitalium* PCR product was 282 bp. Using designed primers and extracted DNA from cervical specimens, PCR was conducted. PCR reaction was done in a total volume of 25 μL of PCR master mix (CinnaGen, Tehran, Iran). The PCR amplification program in a Thermocycler (Eppendorf, Hamburg, Germany) was: initial denaturation 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 64°C for 30 seconds, and extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. PCR products were separated by gel electrophoresis apparatus (GE-100, Shanghai Beiyi Bioequip Information Co., Ltd 906 Lianye Building, No. 299 South Wulumuqi Road, Shanghai, 200031, P.R. China) in 1.5% gel agarose, stained by ethidium bromide, and visualized by UV light and then photographed. The PCR positive control was DNA extracted from isolated *M. genitalium*.

2.4. Statistical analysis

The data were entered into SPSS 16.0 statistical software (SPSS, Chicago, IL, USA), and analyzed. Results were summarized and shown as tables and percentages. Fisher test was used for comparison of qualitative variables.

3. Results

The prevalence of *M. genitalium* infection was one (0.91%) in the patient group (spontaneous abortion) and

Table 1. Demographic data and history of risk factors and prevalence of *Mycoplasma genitalium* infection in 109 women with spontaneous abortion (patient group) and 109 women with normal delivery (control group).

Variables	Patients <i>n</i> = 109	Controls <i>n</i> = 109	<i>p</i>
Age (y), mean \pm standard deviation	29.6 \pm 5.9	27.8 \pm 4.87	0.83
<i>Mycoplasma hominis</i> in women aged < 25 y	0 (0%)	2 (1.83%)	
<i>M. hominis</i> in women aged \geq 25 y	2 (1.83%)	2 (1.83%)	
Education			0.08
Illiterate	5 (4.58%)	3 (2.75%)	
Primary	49 (44.95%)	33 (30.27%)	
High school	36 (33.02%)	44 (40.36%)	
University	19 (17.43%)	29 (26.60%)	
Occupation			1
Housekeeper	97 (88.99%)	97 (88.99%)	
Employee	12 (11.00%)	12 (11.00%)	
History of smoking	0 (0%)	3 (2.75%)	0.43
History of preterm premature membrane rupture before 37 wk gestation	5 (4.58%)	1 (0.91%)	0.084
History of vaginal infection	11 (10.09%)	5 (4.58%)	0.115
History of urinary infection	9 (8.25%)	8 (7.33%)	0.801
Prevalence of <i>M. hominis</i> infection	2 (1.83%)	4 (3.66%)	0.68

three (2.75%) in the control group (normal pregnancy; Table 1). The age of the women ranged from 19 years to 43 years (29.6 ± 5.9 years) in the patient group and from 19 years to 42 years (27.8 ± 4.87 years) in the control group. Parity was two in the patients and control groups and both groups had two children (average number). Urinary and vaginal coinfections in the patient and control groups were observed in two and zero individuals, respectively (Table 1). The frequency of urinary tract infection in the husbands of patients was two, but zero in the control group. The frequency of smoking was three women in the patient group and three in the control group, and the frequency of alcohol consumption was zero in both groups. Demographic information and other patient information is presented in Table 1. A representative stained gel following PCR assay is shown in Figure 1. Using Fisher test, the association between *M. genitalium* infection and spontaneous abortion was found to be nonsignificant (odds ratio, 0.32; confidence interval, 0.03–0.19; $p = 0.62$).

4. Discussion

Available data on the role of *M. genitalium*, infections in pregnancy adverse outcomes is increasing, but its role in spontaneous abortion is controversial. Dehon and McGowin [15], using real-time quantitative PCR reported that in 473 women with a low risk for acquiring STDs, the prevalence rate of *M. genitalium* was 1.5%. In our study, in patient and control groups, 10.09% and 4.58% of women had vaginal infections, respectively. Studies showed that *M. genitalium* is a common causative agent of STDs in men and women

and is a reason for infertility among infected people [16]. In Iran, Amirmozafari et al [17] reported that in 210 genital swabs taken from 210 patients, mycoplasma strains were isolated from 39.5% of patients using a selective mycoplasma isolation media, but using PCR 57.1% of patient samples were found to be positive for mycoplasmas. In our study, PCR showed that the total prevalence of *M. genitalium* infection was four (3.66%) in patient and control groups. PCR is highly sensitive because genus specific primers are used for diagnosis of genital mycoplasmas in comparison with culture [17]. In Cuba, Mondeja et al [18] using a culture method in Vero cell suspensions, 16S ribosomal RNA, and MgPal–3 PCR showed that all 11 isolates that were detected as *M. genitalium* with the culture method were also positive for *M. genitalium* with PCR [18]. Usually, PCR is used to detect *M. genitalium* infections, because it has proved to be more sensitive, specific, and rapid than the conventional microbiology methods [8,19]. For example, detection of *M. genitalium* by the culture method requires up to 8 weeks [9,20].

A study reported *M. genitalium* infections in symptomatic and asymptomatic men referred to the Urology Clinic in Tehran, Iran. The prevalence of *M. genitalium* was 12% and 2% in symptomatic and asymptomatic men, respectively, and *M. genitalium* infection was associated with dysuria, testis inflammation, pelvic pain, and low educational level [4]. In Mirnejad et al's [20] study, genital swabs were taken from 210 women in Tehran, Iran, and, using PCR the frequency of *M. genitalium* was 3.3%. The prevalence of *C. trachomatis* and *M. genitalium* in pregnant women of Sabzevar (north-east of Iran) in Haghighi Hasanabad et al's [9] study was determined in 196 urine specimens by duplex PCR. A total of 31 (15.81%) specimens were positive, with *C. trachomatis* in 27 (13.7%), *M. genitalium* in two (1.02%), and coinfection with both in two (1.02%) specimens. Also, significant correlation was found between preterm labor and infection [9]. In our results, prevalence of *M. genitalium* in patient and control groups were 0.91% and 2.75%, respectively. The results of *M. genitalium* prevalence in the aforementioned studies are nearly similar to our study. Differences of the results in some studies may be due to the type of population (pregnant vs. nonpregnant), sample types (urine vs. swab), date of study (recent vs. past), as well as the detection methodologies [1,21,22].

Among cervical samples from 1,014 women in a case–control study, 6.2% were infected with *M. genitalium* in Guinea-Bissau using PCR assay; *M. genitalium* did not have a deleterious impact on the outcome of pregnancy [21]. In a prospective cohort study of 877 women with singleton pregnancies at < 11 weeks of gestation *M. genitalium* was examined in vaginal swabs by PCR in Japan. *M. genitalium* were detected in 0.8%, of the women. No significant association was found between preterm labor and *M. genitalium* [5], which is

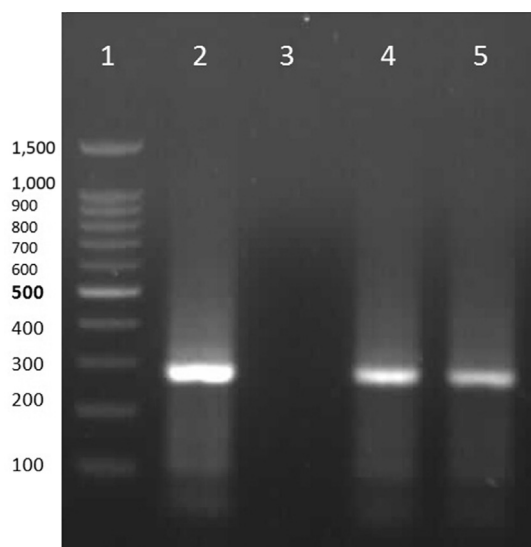


Figure 1. Polymerase chain reaction (PCR) test for *Mycoplasma genitalium* detection: Lane 1, 100-bp DNA Ladder (CinnaClon, Tehran, Iran); Lane 2, PCR positive control; Lane 3, negative control; Lanes 4 and 5, positive PCR products (282 bp).

compatible with our findings: there was no association between *M. genitalium* infection and spontaneous abortion in our results. The lack of association in our study may be explained by health promotion in the population, and failure to identify potential confounding factors. However, Capoccia et al [8] showed that the role of *Mycoplasma* spp. and *U. urealyticum* in adverse pregnancy outcomes is increasing. In our study, history of vaginal infection in the patient group was somewhat more than control group, but this was not statistically significant. The presence of this microorganism in the vaginal flora may be insufficient to cause pathological problems, but combined with other factors such as other bacterial vaginosis or cervical incompetence may also induce spontaneous abortion. However, a screening program is important in sexually active women and needed as part of case-finding strategies and treatment. Whether detection and antimicrobial therapy of mycoplasma in pregnant women can effectively reduce the incidence of unwanted pregnancy outcomes needs further investigation.

In conclusion, our study showed that *M. genitalium* may be positive in the genital tract of pregnant women but was not associated with spontaneous abortion. The low prevalence of *M. genitalium* and no association with pregnancy adverse outcome suggests that is unlikely to be an important risk factor in healthy women. Further powerful studies with larger sample sizes are needed for determination of possible role of *M. genitalium* in reproductive, pregnancy outcomes. Such studies should detect *M. genitalium* in cervical or vaginal specimens from women prior to pregnancy or early in gestation.

Conflicts of interest

All authors have no conflicts of interest to declare.

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